Inhibitory Effect of Garlic, Clove and Carrot on Growth of *Aspergillus Flavus* and Aflatoxin Production

Dusanee Thanaboripat Kowit Nontabenjawan Kwanruen Leesin Detchpol Teerapiannont
Oratai Sukcharoen Rararat Ruangrattanamatee
Department of Applied Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.

ABSTRACT The inhibitory effect of crude extracts of garlic, clove and carrot at concentrations of 20,000, 40,000, 60,000, 80,000 and 100,000 μg/mL on growth of *Aspergillus flavus* and aflatoxin production in rice was investigated. The results showed that garlic, clove and carrot could inhibit the growth of *A. flavus* and aflatoxin production. Garlic and clove at 100,000 μg/mL highly reduced the level of aflatoxin from 5.94 to 0.15 and 0.06 μg/g respectively whereas carrot at 20,000μg/mL reduced the most level of aflatoxin from 5.94 to 0.03μg/g. Garlic, clove and carrot at 100,000μg/mL also inhibited the mould growth. The most effective herb for inhibiting the growth of *A. flavus* and aflatoxin production was garlic.

Key words: Inhibitory effect, *Aspergillus flavus* growth, Aflatoxin production

INTRODUCTION

Aflatoxins are toxic secondary metabolites produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (Kurbzman *et al.*, 1987). These fungi can grow readily on a wide variety of agricultural commodities and pose health hazard to both human and animals. Aflatoxins are acutely toxic, carcinogenic, teratogenic and mutagenic (Goldblatt, 1969; Ciegler, 1975). Various strategies have been proposed for the prevention and detoxification of aflatoxin in foods and feeds. However, preventing the contamination of food and feed by the toxigenic fungi is the most rational and economic approach to avoid the potential hazards (Masood and Ranjan, 1990; Samarajeewa *et al.*, 1990). Powders and extracts of many herbs, spices and essential oils have been reported to have antimicrobial activity and some of them also inhibit aflatoxin formation in synthetic media (Bahk and Marth, 1983; Madhyastha and Bhat, 1984; Farag *et al.*, 1989; Sinha *et al.*, 1993; Masood *et al.*, 1994; Prasad *et al.*, 1994).

The present study was undertaken to determine the effect of crude extracts of garlic, clove and carrot on growth of *A. flavus* and aflatoxin production in rice.

MATERIALS AND METHODS

Organism *A. flavus* 102566 obtained from Commonwealth Mycological Institute (CMI), U.K. was used throughout this study.

Preparation of Spore Suspension *A. flavus* was grown on potato dextrose agar slant at 30 ℃ for 7 days. Spores were harvested by adding sterile distilled water plus 0.05% Tween 80. The spore suspension was pooled in a sterile bottle and the number of spores counted by an improved Neubrauer Haemacytometer.

Herbs Garlic, clove and carrot purchased from a local market in Bangkok were cleaned and blended with the addition of distilled water to the acquired concentration. The blended herb was then extracted into distilled water. The extracted solution was then filtered and centrifuged at 9,000 g. The supernatant was refiltered through a Whatman filter No. 1 and sterilized by membrane filtration technique using membrane filter with the diameter of 0.45 μm. The filtrate was used as test extract.

Media Malt extract agar contained 30 g of malt extract, 5 g of mycological peptone and 15 g of agar suspended in 1 L of distilled water and rice agar contained 200 g of rice and 15 g of agar suspended in 1 L of distilled water were sterilized and used for the experiment.

Culture Conditions Effect of herbs on growth of *A. flavus* in malt extract and rice agars A colony of *A. flavus* 102566 was grown on malt extract agar (MEA) for 7 days at 30 ℃. Cork borer with the diameter of 0.4 cm was used to cut the edge of the colony into a disc and transferred to the center of a petri dish containing MEA and rice agar with herbs at various concentrations, i. e., 0, 20,000, 40,000, 60,000, 80,000, and 100,000 μ g/mL. The cultures were then incubated for 7 days at room temperature and the diameters of the colonies were measured. Each experiment was replicated 4 times.

Effect of herbs on growth of *A. flavus* and aflatoxin production in rice Fifty grams of rice were sterilized and added with various concentrations of herbs. The initial moisture content of the substrate was adjusted to 23% by adding sterile distilled water and the spores of *A. flavus* (10^6) spores/g) were inoculated. Cultures were then incubated at room temperature for 7 days. Each experiment was replicated 4 times.

Aflatoxin Analysis The sample was extracted for aflatoxin by the Sep Pak method (Matsubara, 1985). Sample (50 g) was extracted with 150 mL of chloroform and 25 mL of distilled water in a shaker at 250 rpm for 30 min. The extract was then filtered through Whatman No. 1 filter paper with the aid of Celite and the chloroform layer was separated from the filtrate. The filtrate was washed once again with 50 mL of chloroform and the chloroform extracts were pooled together and evaporated to dryness. The dried extract was dissolved with 10 mL of chloroform-hexane (3:7) and then added to the Sep Pak silica gel cartridge column. The column was drained and eluted with 10 mL of hexane followed by 10 mL of benzene-acetic acid (95.5:4.5) and 10 mL of ethyl ether-hexane (60:40). The eluate was discarded and aflatoxin was eluted with 15 mL of methylene chloride-acetone (9:1) and evaporated to dryness. The eluate was dissolved with 1 mL of methanol, filtered through 0.45 μm membrane filter (Millipore) and 10 μl injected to the HPLC (Shimadzu) using the following conditions: flow rate of 1 mL/min., UV spectrophotometric detector at 365 nm, reverse phase column C18 and mobile phase solvent (water:acetic acid=1:1). The amount of aflatoxin was calculated from the chromatogram by comparison to the standard aflatoxin chromatogram.

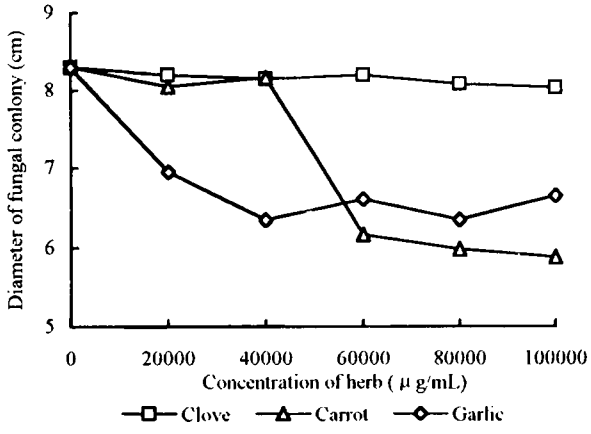

Fig. 1 Effect of herbs on growth of *A. flavus* in malt extract agar

Statistical Analysis The analysis of variance (ANOVA) at significant difference of 0.01 and Duncan's multiple range test (Montgomery, 1984) were calculated to indicated the effect of herbs on growth of *A. flavus* and aflatoxin production.

RESULT AND DISCUSSION

The effect of garlic, clove and carrot on growth of *A. flavus* in MEA and rice agar are shown in Figs. 1 and 2. The results showed that garlic and carrot had better inhibitory effect on growth of *A. flavus* than clove. Carrot showed to be the best herb for controlling the growth of this fungus at concentrations of 60,000-100,000 μg/mL. Statistical analyses on the effects of these herbs on growth of *A. flavus* are given in Table 1, 2, 3 and 4. The growth of *A. flavus* in MEA was significantly reduced by garlic and carrot but no significant reduction was found with clove treatment (Table 1).

Table 1. Analysis of variance on the effect of herbs on growth of *A. flavus* in malt extract agar

SOV	df	SS	MS	F
H	2	22.91	11.46	
C/garlic	5	10.84	2.17	30.97*
C/clove	5	0.18	0.04	0.57
C/carrot	5	28.29	5.66	80.86*
Error	54	3.08	0.07	
Total	71	66.02		

Garlic at 40,000 μg/mL and carrot at 60,000 μg/mL and above gave the best reduction of fungal growth (Table 2).

Table 2. Multiple comparison test on the effect of herbs at various concentrations on growth of *A. flavus* in malt extract agar

Concentration	0	20,000	40,000	60,000	80,000	100,000
Garlic	8.30^{c}	6.96^{b}	6.35^{a}	6.61^{ab}	6.35^{a}	6.65^{ab}
Clove	8.30^{b}	8.20^{b}	8.15^{b}	8.20^{a}	8.08^{a}	8.03^{a}
Carrot	8.30^{a}	8.04^{a}	8.16^{a}	6.16^{a}	5.98^{a}	5.88^{a}

When the fungus was grown in rice agar, significant reduction in the fungal growth was found with all treatment (Table 3).

Table 3. Analysis of variance on the effect of herbs on growth of *A. flavus* in rice agar

SOV	df	SS	MS	F
H	2	20.13	10.06	
C/garlic	5	7.97	1.59	22.71*
C/clove	5	1.25	0.25	3.57*
C/carrot	5	36.45	7.29	104.14*
Error	54	4.02	0.07	
Total	71	69.82		

Garlic at 40,000 μg/mL and carrot at 60,000 μg/mL and above gave the best reduction of fungal

growth whereas clove at 20,000 and 40,000 μg/mL gave the best reduction (Table 4).

Table 4. Multiple comparison test on the effect of herbs at various concentrations on growth of *A. flavus* in rice agar

Concentration	0	20,000	40,000	60,000	80,000	100,000
garlic	7.95^{c}	7.14^{b}	6.60^{a}	6.69^{ab}	6.36^{a}	6.24^{a}
clove	7.95^{b}	7.77^{ab}	7.38^{a}	7.90^{b}	8.03^{b}	8.05^{b}
carrot	7.95^{b}	7.91^{b}	7.74^{b}	5.23^{a}	5.35^{a}	5.68^{a}

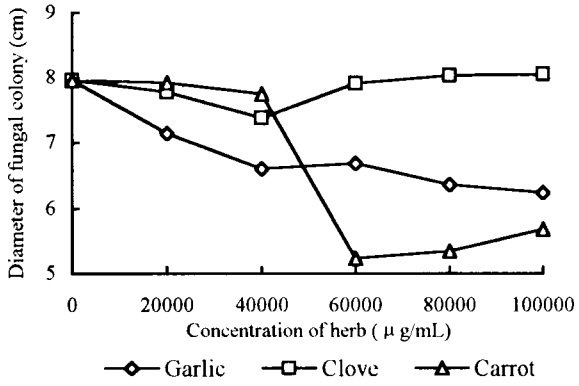

Fig. 2 Effect of herbs on growth of *A. flavus* in rice agar

The effects of garlic, carrot and clove on aflatoxin production in rice agar are given in Fig. 3. All herbs at all concentrations inhibited the aflatoxin production. Statistical analyses on the effect of these herbs on aflatoxin production are shown in Table 5 and 6. Aflatoxin was significantly reduced by all treatments. Garlic and clove at 40, 000 μg/mL and above and carrot at 20,000-60,000 μg/mL gave the most inhibitory effect.

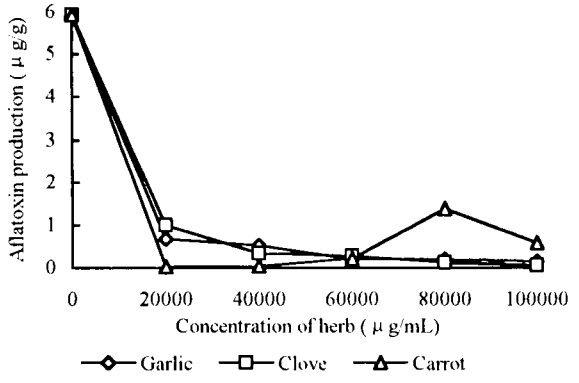

Fig. 3 Effect of herbs on aflatoxin production by *A. flavus* in rice

Table 5. Analysis of variance on the effect herbs on aflatoxin production by *A. flavus* in rice

SOV	df	SS	MS	F
H	2	0.10	0.05	
C/garlic	5	105.10	21.02	420.40^{a}
C/garlic	5	106.02	21.22	424.40^{a}
C/carrot	5	105.69	21.13	422.60^{a}
Error	54	2.55	0.05	
Total	71	319.45		

Inhibition in the growth of aflatoxin producing fungi and aflatoxin formation by garlic, clove and carrot has been well reported. Graham and Graham (1987) found that mycelial growth and toxin production by *A. parasiticus* were inhibited by garlic concentration of 0.3-0.4%. Clove oil also exhibited similar influence on the growth of *A. flavus* and *A. parasiticus* and aflatoxin production if used in sufficient amount (Bullerman et al. ,1977; Farag et al., 1989; Sinha et al., 1993). Batt and colleagues (1980) found that raw carrot tissue did not support the growth of *A. parasiticus* spores.

Table 6. Multiple comparison test on the effect of herbs at various concentrations on aflatoxin production of *A. flavus* in rice

Concentration	0	20,000	40,000	60,000	80,000	100,000
garlic	5.94^{c}	0.67^{b}	0.52^{ab}	0.20^{a}	0.20^{a}	0.15^{a}
clove	5.94^{c}	1.00^{b}	0.33^{a}	0.28^{a}	0.13a	0.06^{a}
carrot	5.94^{d}	0.03^{a}	0.04^{a}	0.21^{ab}	1.38^{c}	0.58^{b}

Essential oils of certain spices and herbs contain antimicrobial substances (Garrido et al., 1992). Thus, some herbs such as garlic, clove and carrot could be applied practically as mold inhibitors in foods and feeds and especially for the prevention of aflatoxin producing fungi.

REFERENCES

1. Batt, C., M. Solberg, M. 1980. Ceponis. Inhibition of aflatoxin production by carrot root extract. J. Food Sci. 45, 1210-1213.

2 Bahk, J., E.H. Marth. 1983. Aflatoxin production is inhabited by selected herbal drugs. Mycopathol. 83, 129-134

3. Bullerman, L.B., F.Y. Lieu, S.A. Seier. 1977. Inhibition of growth and aflatoxin production by cinnamon and clove oils. Cinnamic aldehyde and eugenol. J. Food Sci. 42, 1107-1109

4. Ceigler, A. 1975. Mycotoxins: occurrence, chemistry, biological activity. Lloydia. 38, 21-35

5. Farag, R.S., Daw, Z.Y., Abo-Raya, S.H. 1989. Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. J. Food Sci. 54, 74-76

6. Garrido, D., M. Jodral, R. Pozo. 1991. Mold flora and aflatoxin-producing strains of *Aspergillus flavus* in spices and herbs. J. Food Prot. 55, 451-453

7. Goldblatt, L.A. Aflatoxin. 1969. Academic Press Inc. (London) Ltd., London

8. Graham, H.D., E.J.F. Graham. 1987. Inhibition of *Aspergillus parasiticus* growth and toxin production by garlic. J. Food Safety. 8, 101-108

9. Kurtzman, C.P., B.W. Horn, C.W. Hesseltine. 1987.

Aspergillus nomius, a new aflatoxin producing species related to *Aspergillus flavus* and *Aspergillus tamarii*. Antonie van Leeuw. 53, 147-158.

10. Madhyastha, M.S., R.V. Bhat. 1984. *Aspergillus parasiticus* growth and aflatoxin production on black and white pepper and the inhibitory action of their chemical constituents. App. Env. Microbiol. 48, 568-571

11. Masood, A., J.V.V. Dogra, A.K. Jha. 1994. The influence of coloring and pungent agents of red chilli (*Capsicum annum*) on growth and aflatoxin production by *Aspergillus flavus*. Lett. App. Microbiol. 18, 184-186

12. Masood, A., K.S. Ranjan. 1990. The influence of fungitoxicants on growth and aflatoxin production by *Aspergillus flavus*. Lett. App. Microbiol. 11, 197-201

13. Matsubara, I. 1995. Some remarks on aflatoxin problems. Seminar on aflatoxin, Export Training Center, Department of Commercial Relations, Bangkok. p8, March 8

14. Montgomery, D.C. 1984. Design and analysis of Experiments. John Wiley and Sons, Inc., New York

15. Prasad, G., S.S. Sahay, A. Masood. 1994. Inhibition in aflatoxin biosynthesis by the extract of *Amorphophallus campanulatus* (OL) and calcium oxalate. Lett. App. Microbiol. 18, 203-205

16. Samarajeewa, U., A.C. Sen, M.D. Cohen, C.J. Wei. 1990. Detoxification of aflatoxin in foods and feeds by physical and chemical methods. J. Food Prot. 53, 489-501

17. Sinha, K.K., A.K. Sinha, G. Prasad. 1993. The effect of clove and cinnamon oil on growth of aflatoxin production by *Aspergillus flavus*. Lett. App. Microbiol. 16, 114-117

(**Responsible Editor**: Sun Yueqi)